

in the specification, in the Nehme Declaration and in the enclosed graphs as “tazarotene”) has synergistic effects in three colon cancer cell lines with the chemotherapeutic agent 5-fluorouracil (5-FU), and in four separate colon cancer cell lines with the chemotherapeutic agent and 7-ethyl-10-hydro-(20)S-camptothecin (also known as SN-38 or CPT11). The nature of the assays wherein these results were obtained is also described in the Nehme declaration.

Instant Claims 1 and 14 were amended to set forth that the disease or condition being treated is breast cancer, colon cancer or leukemia. The last Office Action has already recognized that treatment of breast cancer and leukemia is supported by the data contained in the specification. As is explained above and shown by the Nehme Declaration, treatment of colon cancer in accordance with the invention is also supported by data. The concept that colon cancer can be treated in accordance with the present invention is noted in the originally filed specification that refers to “solid tumors”, and also in line 1 page 2, where it is noted that retinoids are generally useful for treating tumors of the colon. (Tazarotene being a retinoid the statement in line 2, page 2 is clearly applicable to it.)

The Office Action indicated that Claims 3, 4, 7, 8, 11, 12, 17, 18, 22, 23, 28 and 29 are allowable if the issue of being “substantial duplicates” is resolved.

To simplify review of these claims and to avoid excessive “amendments” these claims were canceled and Claims 31 through 45 were substituted in their place.

Specifically, new independent Claim 31 is drawn to a composition for the treatment of breast cancer and leukemia, combining the subject matter of Claims 3 and 4. Its dependent Claim 32 is drawn to treatment of breast cancer and leukemia in combination with the specific chemotherapeutic agent interferon.

Claim 33 is drawn to a composition of a different scope for the treatment of breast cancer and leukemia, combining the subject matter of Claims 7 and 8. Its dependent Claim 34 is drawn to treatment of breast cancer and leukemia in combination with the specific chemotherapeutic agent interferon.

Claim 35 is drawn to a composition of still different scope for the treatment of breast cancer and leukemia, combining the subject matter of Claims 11 and 12. Its dependent Claim 36 is drawn to treatment of breast cancer and leukemia in combination with the specific chemotherapeutic agent interferon.

Method of treatment Claim 37 is drawn to using a composition for the treatment of breast cancer and leukemia, combining the subject matter of Claim 17 and 18,. Its dependent Claim 38 is drawn to treatment of breast cancer and leukemia in combination with the specific chemotherapeutic agent interferon. Dependent claim 39 specifies human recombinant interferon  $\alpha$ , human recombinant interferon  $\beta$ , or human recombinant interferon  $\gamma$  as the other chemotherapeutic agent.

Method of treatment Claim 40 is drawn to using a composition of a different scope for the treatment of breast cancer and leukemia, combining the subject matter of Claim 22 and 23. Its dependent Claim 41 is drawn to treatment of breast cancer and leukemia in combination with the specific chemotherapeutic agent interferon. Dependent claim 42 specifies human recombinant interferon  $\alpha$ , human recombinant interferon  $\beta$ , or human recombinant interferon  $\gamma$  as the other chemotherapeutic agent.

Method of treatment Claim 43 is drawn to using a composition of a still different scope for the treatment of breast cancer and leukemia, combining the subject matter of Claim 28 and 29. Its dependent Claim 44 is drawn to treatment of breast cancer and leukemia in combination with the specific chemotherapeutic agent interferon. Dependent claim 45 specifies human recombinant interferon  $\alpha$ , human recombinant interferon  $\beta$ , or human recombinant interferon  $\gamma$  as the other chemotherapeutic agent.

The above-discussed newly added claims in effect replace allowable claims 3, 4, 7, 8, 11, 12, 17, 18, 22, 23, 28 and 29 and also resolve the issues of “substantial duplication”.

In light of the foregoing all outstanding claims are in *prima facie* allowable condition, and their early allowance is respectfully solicited.

Supplemental Information Disclosure

Simultaneously with the filing of the present amendment, applicant submits a Supplemental IDS with references that were cited less than 3 months ago in an International Search Report of the corresponding PCT application.

Change of Address

On July 12, 2002 applicant's undersigned attorney filed a PTO/SB/122 Change of address form pertaining to this case. A copy of this form is enclosed. Office personnel is respectfully requested to take notice of the undersigned attorney's current address indicated on the form, and also indicated below.

In accordance with the Patent Office Rules effective March 1, 2001, in the appended attachment titled "Version with Markings to Show Changes Made" applicant, acting through the undersigned attorney, also presents the amended subject matter in the format wherein the deleted material is indicated in square brackets and added material is underlined in accordance with prior practice.

In the event the Examiner is of the opinion that a telephone conference with the undersigned attorney would materially facilitate the final disposition of this case, he is respectfully requested to telephone the undersigned attorney at the below listed telephone number.

Respectfully submitted

By:

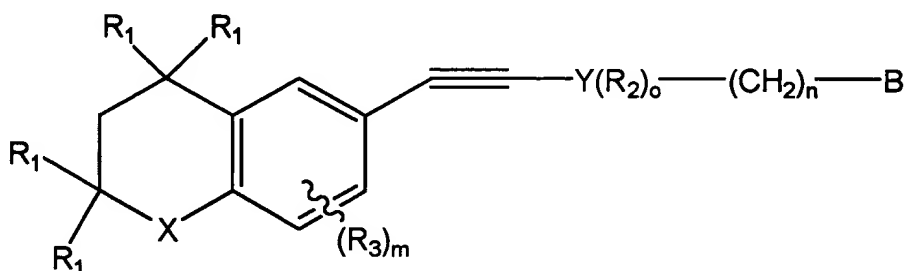
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

**Claim 1 (twice amended)**

A pharmaceutical composition for the treatment of a malignant disease or condition in a mammal, said condition being selected from the group consisting of breast cancer, colon cancer and leukemia, the composition comprising a pharmaceutically acceptable excipient and a therapeutically effective dose of a compound of the formula



where **X** is S or O;

**R<sub>1</sub>** is, independently, H or lower alkyl of 1 to 6 carbons;

**R<sub>2</sub>** and **R<sub>3</sub>** are, independently, H, lower alkyl of 1 to 6 carbons, F, Cl, Br, I, alkoxy of 1 to 6 carbons, or fluoroalkoxy of 1 to 6 carbons;

**m** is an integer 0 to 3;

**o** is an integer 0 to 4;

**n** is 0-5;

**Y** is phenyl, naphthyl, or a heteroaryl group selected from a group consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl; oxazolyl, thiazolyl, or imidazolyl; and

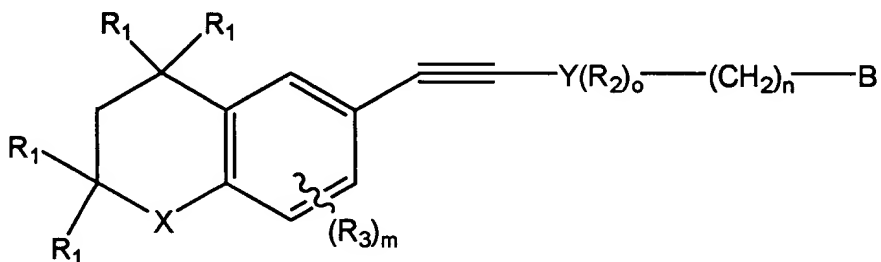
**B** is COOH, a pharmaceutically acceptable salt thereof, CONR<sub>6</sub>R<sub>7</sub> or COOR<sub>8</sub> where **R<sub>6</sub>** and **R<sub>7</sub>**, independently, are hydrogen or an alkyl group of 1 to 6 carbons and **R<sub>8</sub>** is alkyl of 1 to 6 carbons,

said composition being adapted to be used in combination with another chemotherapeutic agent effective for the treatment of the malignant disease or condition of the mammal where the composition in combination with the other chemotherapeutic agent shows synergistic effect.

**Claim 14 (twice amended)**

A method of treating a malignant disease or condition in a mammal in need of such treatment, said condition being selected from the group consisting of breast cancer, colon cancer and leukemia, the method comprising the steps of:

administering to said mammal a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective dose of a compound of the formula



where  $X$  is S or O;

$R_1$  is, independently, H or lower alkyl of 1 to 6 carbons;

$R_2$  and  $R_3$  are, independently, H, lower alkyl of 1 to 6 carbons, F, Cl, Br, I, alkoxy of 1 to 6 carbons, or fluoroalkoxy of 1 to 6 carbons;

$m$  is an integer 0 to 3;

$o$  is an integer 0 to 4;

$n$  is 0-5;

$Y$  is phenyl, naphthyl, or a heteroaryl group selected from a group consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl; oxazolyl, thiazolyl, or imidazolyl;

**B** is COOH, a pharmaceutically acceptable salt thereof, CONR<sub>6</sub>R<sub>7</sub> or COOR<sub>8</sub> where R<sub>6</sub> and R<sub>7</sub>, independently, are hydrogen or an alkyl group of 1 to 6 carbons and R<sub>8</sub> is alkyl of 1 to 6 carbons, and

co-administering to said mammal with said compound another chemotherapeutic agent effective for the treatment of the malignant disease or condition of the mammal where the composition in combination with the other chemotherapeutic agent shows synergistic effect.

# PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

) Examiner: Robert S. Landsman  
) Art Unit: 1647

1

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For: TREATMENT OF TUMORS  
WITH ACETYLENES DI-  
SUBSTITUTED WITH A PHE-  
NYL OR HETEROAROMATIC  
GROUP AND A SUBSTITUTED  
CHROMANYL, THIOCHROMA-  
NYL OR TETRAHYDROQUINO-  
LINYL GROUP IN COMBINA-  
TION WITH OTHER ANTI-  
TUMOR AGENTS

Irvine, California  
February , 2003

### Certificate of Mailing

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Assistant Commissioner of Patents, Washington / D.C. 20231 .

D.C. 202  
Lore Whyte

Toni Whyte  
~~Linda Simpson~~

February 21, 2003  
Date

DECLARATION OF ALISSAR NEHME Ph. D.

I, ALISSAR NEHME HEREBY DECLARE AS FOLLOWS:

1. I have a Ph. D. degree in pharmacology awarded to me in the year 1995 by the University of Toulouse, France. From approximately October 1995 to February 1999 I performed post-doctoral research in the field of pharmacology at the University of California, San Diego, California. Since March 1999 I have been employed in the Retinoid Research Department of Allergan Inc. as a scientist. My work at Allergan relates principally to the pharmacology of retinoid and related compounds.

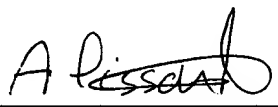
2. I am making this Declaration in connection with and in support of the above-identified application for United States patent.

3. Certain assays and tests were performed by Oncotech Inc. of Irvine California in cooperation with Allergan Inc., to test the drug ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)ethynyl]nicotinate (hereinafter "tezarotene") in combination with the chemotherapeutic agent 5-fluorouracil (5-FU) and 7-ethyl-10-hydro-(20)S-camptothecin (also known as SN-38 or CPT11) in several colon carcinoma cell lines. The nature of the tests or assays is described in **Exhibit A** attached to my Declaration and titled "Materials and Methods". The results of the assays using tezarotene in combination with 5-FU in the colon cancer cell lines HCT15, DLD-1, and HT29 are shown in the graphs marked collectively as **Exhibit B**. The results of the assays using tezarotene in combination with SN-38 in the colon cancer cell lines HCT15, DLD-1, HT29 and SW480 are shown in the graphs marked collectively as **Exhibit C**.

4. I am familiar with the nature of these assays and with the above-noted results obtained therein. Specifically, the graphs of **Exhibits B** and **C** show that tezarotene and the other chemotherapeutic agent, 5-FU and SN-38, respectively, together have a synergistic effect in inhibiting proliferation of these colon cancer lines.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 02-10-03

  
\_\_\_\_\_  
Alissar Nehme Ph. D.

## **MATERIAL AND METHODS**

The study was conducted at Oncotech, Inc. 1791 Kaiser Avenue, Irvine, CA 92614, under a contract from Allergan Inc., 2525 Dupont Drive, Irvine, CA 92612.

The EDR assay was performed as previously described (1,2). Cells were suspended in 0.12% soft agar in complete medium and plated at different cell concentrations (30,000 cells per well in synergy experiments) onto a 0.4% agarose underlayer in 24-well plates. Plating cells on agarose underlayers supports the proliferation only of the transformed cells, ensuring that the growth signal stems from the malignant component of the tumor.

Tazarotene stock solution in DMSO was prepared at 5 mM. The 5 mM stock solution was diluted in cell culture medium to maximum working stock concentrations of 400 - 500  $\mu$ M at + 37°C. This equated to a maximum final concentration of 25  $\mu$ M of Tazarotene in the EDR Assay. Using DMSO as a solvent was recommended by Dr. S. Nagpal (Allergan, Inc.). SN-38 and 5-FU stock solutions were prepared in PBS and pre-diluted in culture medium. Both drugs were added into designated wells at appropriate concentrations. Colon cancer cells were treated with tazarotene at variable concentrations, alone or in combination with SN-38 or 5-FU used at variable concentrations. All experimental points were represented by three separate wells (triplicates). Controls for the effects of the diluent (DMSO) were also included in each experiment. Cells were incubated with drugs under standard culture conditions for 5 days. Cultures were pulsed with tritiated thymidine ( $^3$ H-TdR, New Life

Science Products, Boston, MA) at 5  $\mu$ Ci per well for the last 48 hours of the culture period. Cell culture plates were then heated to 90°C to liquefy the agarose, and cells were harvested onto glass fiber filters, which were then placed into counting vials containing liquid scintillation fluid. The radioactivity trapped on the filters was counted with a Beckman scintillation counter. The fraction of surviving cells was determined by comparing  $^3\text{H}$ -TdR incorporation in treated (experimental points) and untreated (negative control) wells.

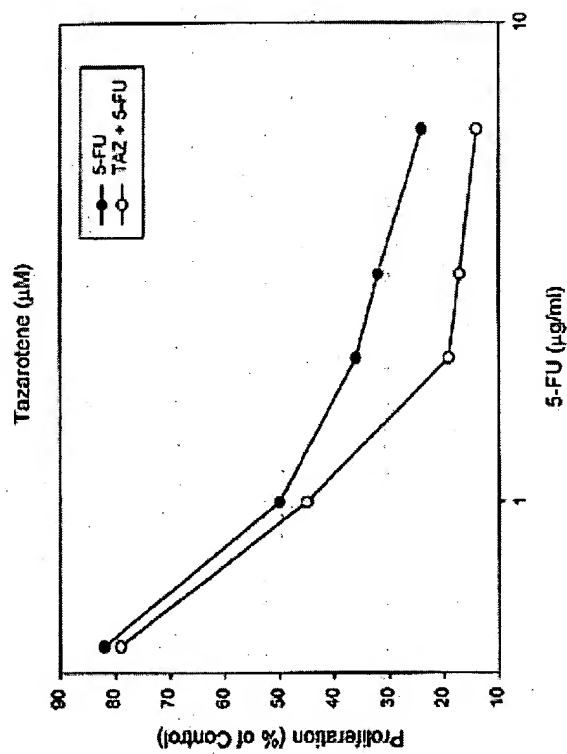
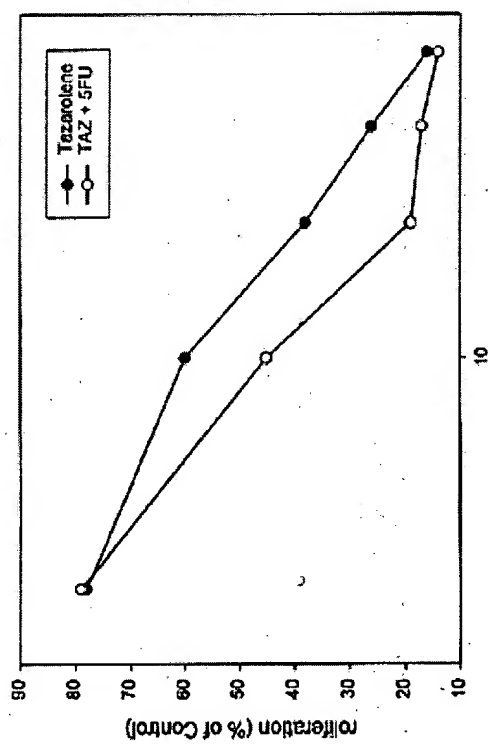
The Chou analysis for drug synergy was performed using dose response curves for the above tumor cell lines as described (3).

## REFERENCES

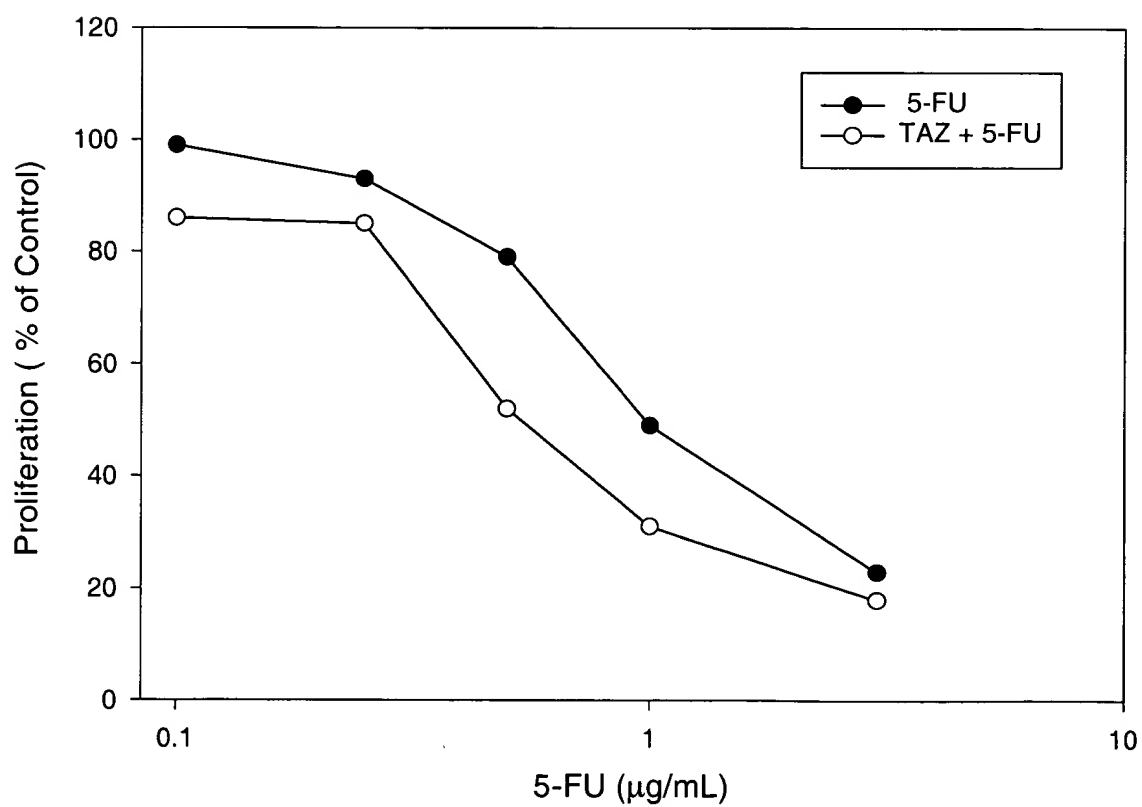
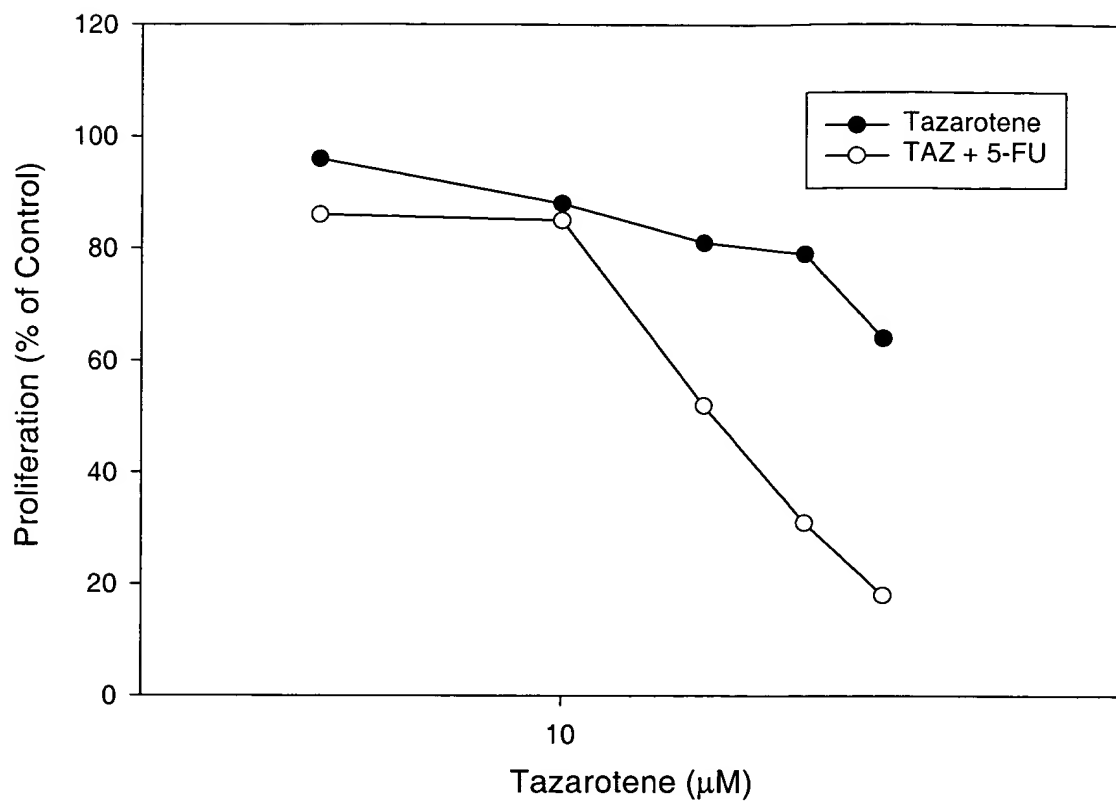
1. Kern DH and Weisenthal LM. Highly specific prediction of antineoplastic drug resistance with an in vitro assay using suprapharmacologic drug exposures. J Nat Cancer Inst. 82:582-588, 1990.
2. Fruehauf JP, Bosanquet AG. In vitro determination of drug response: A discussion of clinical applications. PPO Updates 7(12):1-16, 1993.
3. Chou T.-C. Assessment of synergistic and antagonistic effects of chemotherapeutic agents in vitro. In: Contributions to Gynecology and Obstetrics, O.R. Kohli, B.-U. Sevin, and U. Haller, eds., v.19, pp. 91-107, Karger AG, Basel, Switzerland, 1994.

# Tazarotene Synergy with 5-FU in HCT15 Human Colon Cancer Cells

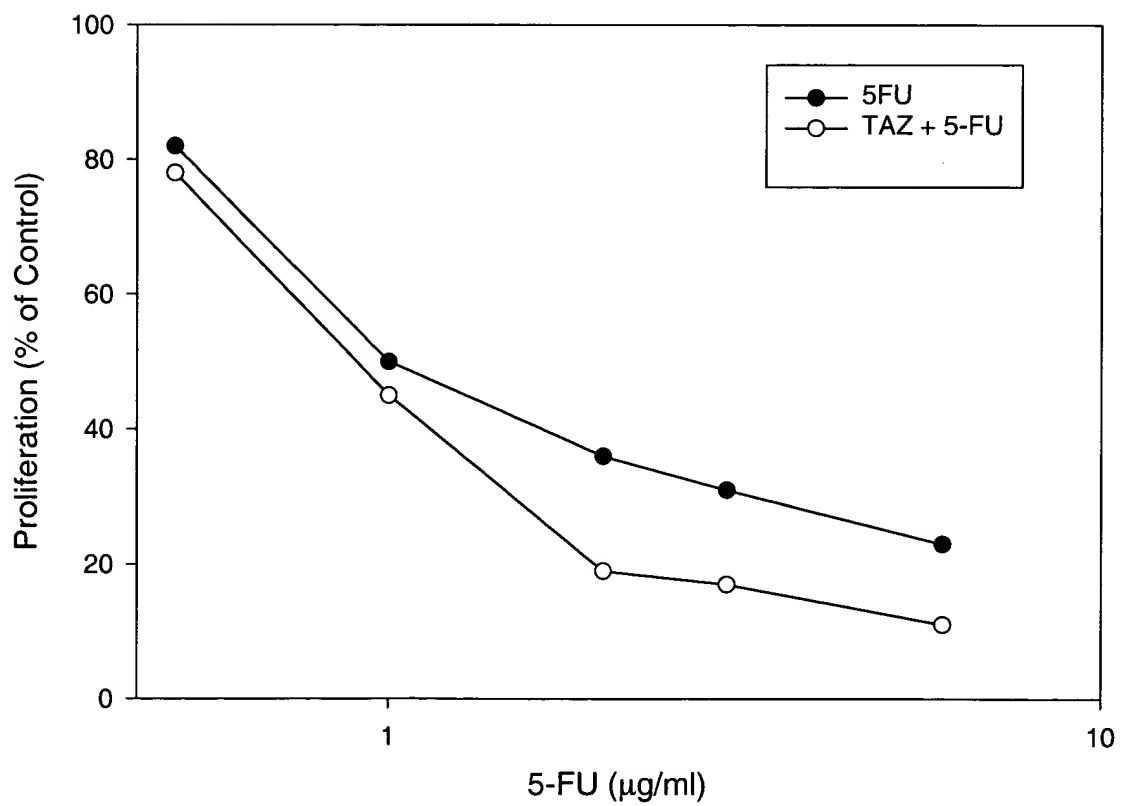
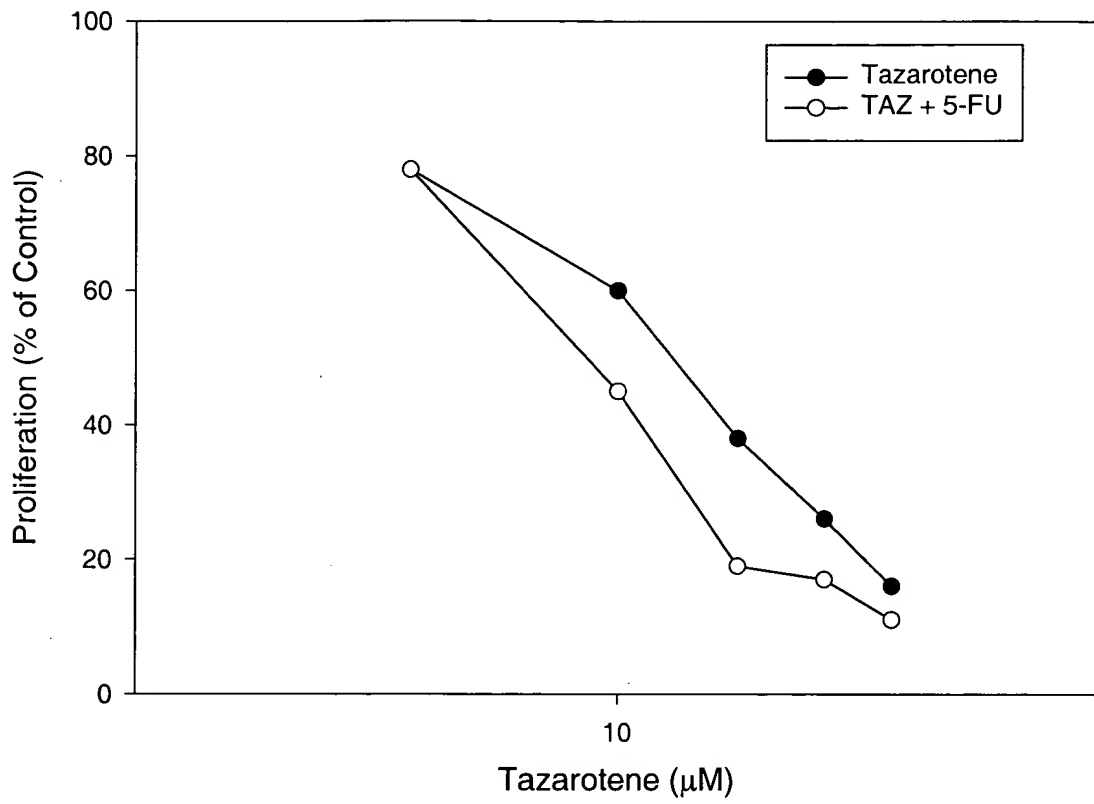
HCT15



# DLD-1

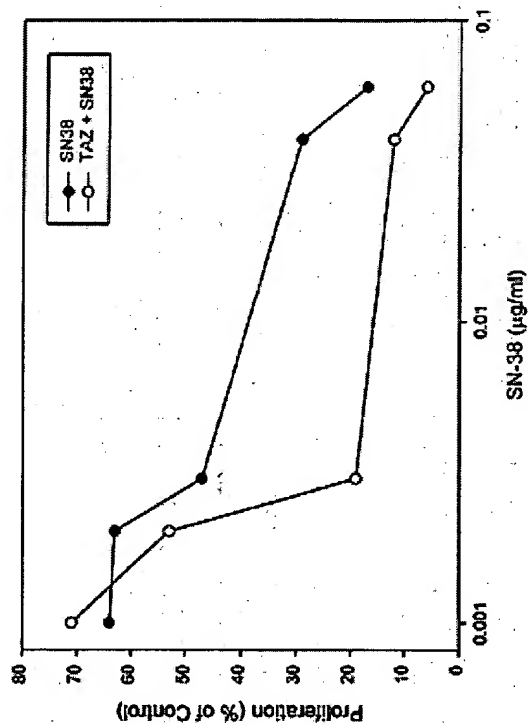
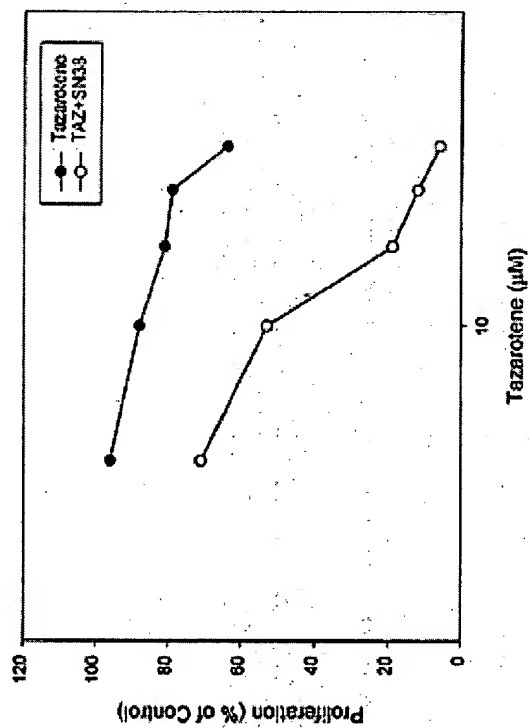


## HT29



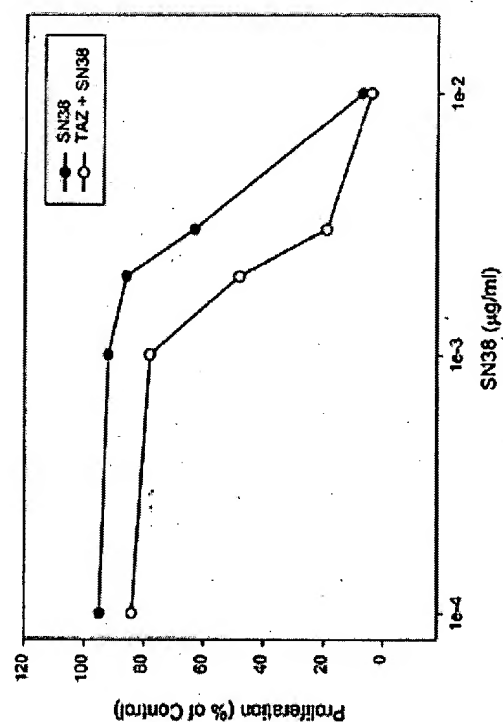
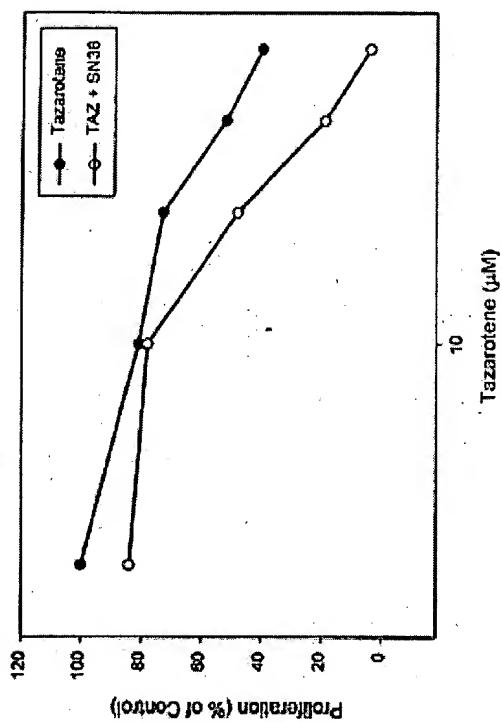
# Tazarotene Synergy with SN-38 in DLD-1 Human Colon Cancer Cells

DLD-1



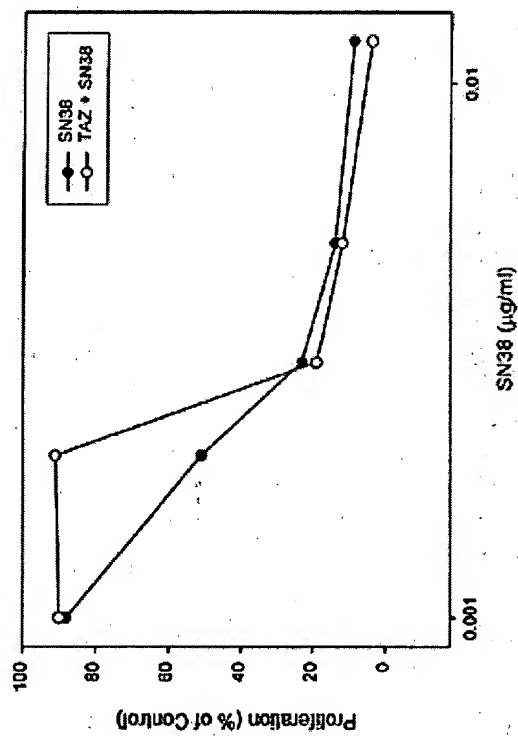
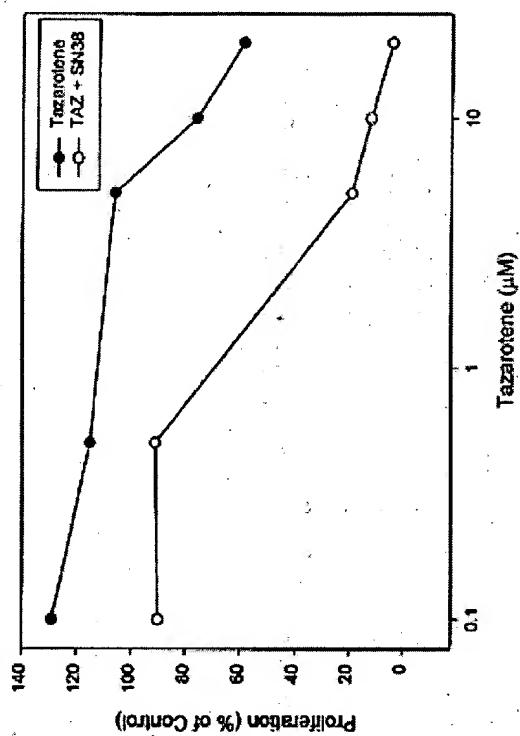
# Tazarotene Synergy with SN-38 in HCT15 Human Colon Cancer Cells

HCT15



# Tazarotene Synergy with SN-38 in HT29 Human Colon Cancer Cells

HT29



# Tazarotene Synergy with SN-38 in SW480 Human Colon Cancer Cells

